O I P E JC10 PATENT & TRADEMARK OFFICE DEC 18 1998

FORM PTO-1390 (REV 12-97) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 3890US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/202104

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/NL97/00345	19 June 1997 (19.06.97)	20 June 1996 (20.06.96)

TITLE OF INVENTION: IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

APPLICANT(S) FOR DO/EO/US: Leonardus Adrianus Maria Govardus VAN LEENGOED, Kasper Hubertus Nicolaas HOEBE, Robert Hans MELOEN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. [X] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [XX] is transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] has been transmitted by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [XX] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [XX] are transmitted herewith (required only if not transmitted by the International Bureau).
   b. [ ] have been transmitted by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [XX] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [XX] Other items or information:

    Preliminary Examination Report
    International Search Report
    Notification of the Recording of a Change

SCANNED5

U S APPLICATION NO (if known, see 37 CFR 1 5)	INTERNATIONAL APPLICATION NO PCT/NL97/00345	ATTORNEY'S DOCKET NUMBER 3890US

17. [X] The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):

Search Report has been prepared by the EPO or JPO $930.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) $720.00

No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $790.00

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1070.00

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $98.00

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [XX] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	70 - 20 =		x $22.00	$ 1,100.00	
Independent claims	3 - 3 =		x $82.00	$ 0	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 2,160.00	
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28). +				$	
SUBTOTAL =				$ 2,160.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$	
TOTAL NATIONAL FEE =				$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 2,000.00	
				Amount to be refunded:	$
				charged:	$

a. [XX] A check in the amount of $ 2,000.00 to cover the above fees is enclosed.

b. [XX] Please charge my Deposit Account No. 20-1469 in the amount of $ 160.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

[XX] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 20-1469 . A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Laurence B. Bond
NAME

30,549
REGISTRATION NUMBER

09/202104
63 Rec'd PCT/PTO 18 DEC 1998

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Loenardus Adrianus Maria Govardus van Leengoed et al.

Serial No.: PCT/NL97/00345

Filed: 19 June 1997

For: IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

Examiner:

Group Art Unit:

Attorney Docket No.: 3890

NOTICE OF EXPRESS MAILING

Express Mail Mailing Label Number:
EL248225768US
Date of Deposit with USPS:
18 December 1998
Person making Deposit:
Jared Turner

PRELIMINARY AMENDMENT

Box
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Please amend the above referenced application as indicated below prior to the calculation of the filing fee.

IN THE CLAIMS:

4. (Amended) A peptide according to claim 1 [, 2, or 3] of 5-20 amino acids.

6. (Amended) A peptide according to claim 1 [,2,3,4, or 5] having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM, QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKSEQATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

7. (Amended) A peptide according to claim 1 [3, 4, or 5] having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

8. (Amended) A peptide composition, wherein at least two peptides according to any of [claims 1-7] claim 1 are chemically linked directly or via spacer molecules.

10. (Amended) A peptide composition wherein at least two peptides according to claim 1 [,2, 3, 4, or 5] having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

11. (Amended) A peptide composition according to claim 8 [,9 or 10] wherein at least four peptides are linked with branching oligolysines.

12. (Amended) A mixture comprising peptides and/or peptide compositions according to [any of claims 1-11] claim 1.

13. (Amended) Antibody specifically directed against a peptide or a peptide composition according to [any of claims 1-11] claim 1.

15. (Amended) A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to [any of the above claims] claim 1 together with at least one suitable excipient for administration.

17. (Amended) Use of a peptide, peptide composition or antibody according to [anyone of claims 1-13] claim 1 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

18. (Amended) A diagnostic assay comprising a peptide [or a peptide composition or an antibody] according to [anyone of claims 1-13] claim 1.

24. (Amended) Use of a peptide [, or peptide composition according to anyone of claims 1-12] claim 1 for the manufacture of a medicament for topical or intra-mammary application.

Please add the following new claims:

25. A peptide according to claim 2 of 5-20 amino acids.

26. A peptide according to claim 25 of 5-12 amino acids.

27. A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

28. A peptide according to claim 2 of 5-20 amino acids.

29. A peptide according to claim 28 of 5-12 amino acids.

30. A peptide according to claim 2 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM, QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR, NFTLKSEQATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

31. A peptide according to claim 2 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

32. A peptide composition, wherein at least two peptides according to claims 2 are chemically linked directly or via spacer molecules.

33. A peptide composition according to claim 32 wherein at least two peptides are linked with lysine.

34. A peptide composition according to claim 32 wherein at least four peptides are linked with branching oligolysines.

35. A peptide composition wherein at least two peptides according to claim 2 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

36. A mixture comprising peptides according to claim 2.

37. Antibody specifically directed against a peptide according to claim 2.

38. Anti-idiotypic antibody raised against an antibody according to claim 37.

39. A pharmaceutical preparation comprising a peptide according to claim 2 together with at least one suitable excipient for administration.

40. Use of a pharmaceutical preparation according to claim 39 in the treatment or prevention of an IL-6 related disease.

41. Use of a peptide according to claim 2 to clear extra-corporeal blood or blood products

from IL-6 or IL-6 receptor molecules.

42. A diagnostic assay comprising a peptide according to claim 2.

43. Use of a diagnostic assay according to claim 42 to detect or diagnose IL-6 related disease in man or animals.

44. Use of a peptide according to claim 31 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 micrograms/ml when tested in vitro in a B9 cell bio assay.

45. Use of a peptide according to claim 44 in a cell-culture.

46. A pharmaceutical preparation comprising a peptide according to claim 31 together with at least one suitable excipient for administration.

47. Use of a pharmaceutical preparation according to claim 6 for topical or intra-mammary application.

48. Use of a peptide according to claim 2 for the manufacture of a medicament for topical or intra-mammary application.

49. A peptide according to claim 3 of 5-20 amino acids.

50. A peptide according to claim 49 of 5-12 amino acids.

51. A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM, QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNEGKKMRCEWDGGR,

NFTLKSEQATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKPNPPHNLSVIN.

52. A peptide according to claim 3 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTTKAVLLLVRKFQNSPAED.

53. A peptide composition, wherein at least two peptides according to claim 3 are chemically linked directly or via spacer molecules.

54. A peptide composition according to claim 53 wherein at least two peptides are linked with lysine.

55. A peptide composition wherein at least two peptides according to claim 3 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

56. A peptide composition according to claim 53 wherein at least four peptides are linked with branching oligolysines.

57. A peptide composition according to claim 55 wherein at least four peptides are linked with branching oligolysines.

58. A mixture comprising peptides and/or peptide compositions according to claim 3.

59. Antibody specifically directed against a peptide or a peptide composition according to claim 3.

60. Anti-idiotypic antibody raised against an antibody according to claim 59.

61. A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to claim 3 together with at least one suitable excipient for administration.

62. Use of a pharmaceutical preparation according to claim 61 in the treatment or prevention of an IL-6 related disease.

63. Use of a peptide, peptide composition or antibody according to claim 3 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

64. A diagnostic assay comprising a peptide according to claim 3.

65. Use of a diagnostic assay according to claim 64 to detect or diagnose IL-6 related disease in man or animals.

65. Use of a peptide according to claim 3 for the manufacture of a medicament for topical or intra-mammary application.

66. Use of a peptide according to claim 52 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 micrograms/ml when tested in vitro in a B9 cell bio assay.

67. Use of a peptide according to claim 66 in a cell-culture.

68. A pharmaceutical preparation comprising a peptide according to claim 52 together with at least one suitable excipient for administration.

69. Use of a pharmaceutical preparation according to claim 68 for topical or intra-mammary application.

70. Use of a peptide according to claim 3 for the manufacture of a medicament for topical

or intra-mammary application.

REMARKS

The Office is respectfully requested to enter the above amendment prior to the calculation of the filing fee.

Respectfully submitted,

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LBB/ll

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§3 Rec'd PCT/PTO 18 DEC 1998

IL-6 AND IL-6-RECEPTOR DERIVED PEPTIDES HAVING IL-6 ANTAGONISTIC OR AGONISTIC ACTIVITY

The invention relates to the field of cytokines. Cytokines are substances that are produced by cells of the immune system and are involved in regulation of humoral and cellular immune reactions and inflammatory responses. Many cytokines are known, and all exert influence on various reactions in the body in a complicated fashion. To illustrate their interdependency and the intricate web of relationships that exist between cytokines, one often speaks about the "cytokine network".

Interleukine 6 (IL-6) is a cytokine which has many effects upon mammalian cells. It exerts these effects through binding to a specific cell surface receptor, that consists of a specific α-subunit of with a molecular weight of approximately 80 kD and a common ß-subunit of approximately 130 kD, also named gp130. The gp130 ß chain is also involved in signal transduction of interleukin-11 (IL-11), leukemia inhibitory factor (LIF), oncostatin M (OM), ciliairy neurotrophic factor (CNTF), and cardiotrophin-1 (CT-1) (P.B. Sehgal, Ling Wang, Ravi Rayanade et al., pp 1-14; volume 762, Annals of the New York Academy of Sciences; 1995).

IL-6 is an extremely pleiotropic cytokine, and its activities include: induction of Ig production by B cells, stimulation of B and T cell growth, differentiation of T cells and macrophages, induction of acute phase protein production by hepatocytes, multilineage hematopoesis, osteoclast formation, maturation of megakaryocytes, and platelet production. IL-6 also effects the central nervous system; IL-6 is an endogenous pyrogen and can induce ACTH production by the pituitary, finally resulting in increased glucocorticoid levels in the circulation. IL-6 exerts its activity by triggering a transmembrane receptor that is present on all target

cells. Specific steps in the IL-6 signaling cascade are the binding to the low affinity α-chain (CD126). The complex of IL-6 and α-chain binds with the high affinity signal transducing ß-chain (GP130, CD130).

In healthy individuals no or only very low levels of IL-6 (<10 pg/ml) are detectable in the circulation. IL-6 levels are increased in various diseases, and it is postulated that these increased levels play a causative role in the pathogenesis of these diseases. Examples of diseases where increased levels of IL-6 are found are multiple myeleoma, AIDS lymphoma, polyclonal B cell activation as observed in AIDS, rheumatoid arthritis, cardiac myxoma and Castleman's disease, mesangial proliferative glomerulonephritis, psoriasis, cancer-associated cachexia, postmenopausal osteoporosis, sepsis, multiple system organ failure, alcohol cirrhosis, and diseases of the central nervous system like Alzheimer, among others. Evidence for the causative role of IL-6 in the pathogenesis of some of the above mentioned diseases has come from from phase I/II clinical trials with IL-6 neutralizing monoclonal antibodies. Treatment with anti-IL-6 monoclonal antibodies reversed fever, acute phase proteins, night sweats, bone destruction, and cachexia. Treatment of a patient with Castleman disease with anti-IL-6 monoclonal antibodies reduced acute phase protein levels, fever, anemia, thrombocytosis, and hypergamma-globulinemia. Improvement of patients was also observed in patients with rheumatoid arthritis. Apparently, reduction of IL-6 activity in these patients resulted in improvement of the clinical signs of their disease.

This approach for treating disease by antagonizing IL-6 activity makes use of monoclonal antibodies directed to IL-6. However, monoclonal antibodies are usually not of human origin and repeated administration of non-human monoclonal antibodie generally leads to immune responses against the constant part of the antibodies, since this is foreign to the body of the patient. This

immunereaction to the monoclonal antibodies used in the treatment is, first of all, counterproductive to the therapeutic treatment itself. The monoclonal antibodies used will be rendered ineffective by the reaction with the antibodies produced by the immune system. Secondly, repeated administration of non-human monoclonal antibodies may elicit such severe immune reactions that they will be detrimental to the patient. Methods for producing less antigenic antibody fragments and methods for humanizing antibodies have been proposed, but, if feasible at all, these methods are not very economical and will their own give rise to problems regarding to half-life and bio-availability. Consequently, using anti-IL-6 monoclonal antibodies in the treatment of IL-6 related disease is considered not to be feasible.

Inhibitors or antagonists based upon mutagenesis of IL-6 have also been proposed, such as IL-6.Q160E /T163P (Brakenhoff, J., de Hon,F., Fontaine, V., et al; J.Biol.Chem.; 269:86-93 (1994)), and IL-6.Q159E/T162P (Ehlers, M., de Hon, D., Klaasse Bos, H, et al., J. Biol. Chem.; 270:8158-8163 (1995)). It has been shown with these mutant proteins that receptor binding of IL-6 and signal transduction of IL-6 can be separated in vitro. However, such mutant proteins are also foreign to the body of the patient to be treated and will also elicit an unwanted and unfavourable immune response that generally is detrimental to the treatment. Furthermore, such mutant proteins may only be partly effective, in that, although they may effectively block or inhibit specific IL-6 activities, at the same time they may exert other effects on the cytokine network with additional, still remaining, reactive sites present on these proteins. Therapeutic treatment with such reagents would then elicit other, yet unpredictable, side effects. A great disatvantage of earlier reported mutant IL-6 and IL-6 receptor antagonists is that these molecules, instead of inhibiting IL-6 in vivo, act as carrier and increase the

half-life time and result in an increase of IL-6 activity in vivo. Moreover, these mutant IL-6 and IL-6 receptor antagonists have a low affinity to their target molecules and will likely act as an immunogen. In addition, antibodies raised to IL-6 stabilize IL-6 and result in an increased IL-6 production. Accumulation of circulating IL-6 as stable IL-6-anti-IL-6 complexes as a result of treatment with these antibodies to IL-6, will occur as no renal filtration can be expected. Repeated use of nonhumanized IL-6 antibodies to human patients will most likely induce antibody production to these antibodies, and result in formation of immune-complexes (Heremans,H., Dillen,C., et al J.Immunol. 22,2395-2401).

The present invention provides a solution to the above illustrated problems without hampering the possibility of therapeutic treatment of IL-6 related disease. The above methods to inhibit IL-6 activity by antibodies or mutants, differ greatly from the invention as described here: peptides that antagonize or agonize IL-6 at the binding site to the receptor in three ways: at the IL-6 part, at the α-receptor part, and at the gp130-receptor part. These antagonists and agonists and combinations of these antagonists and/or agonists as multimeric peptides or as single peptides with defined pharmacokinetic characteristics gives a powerful tool to manage IL-6 bioactivity. With the solution provided by the present invention, immune responses to the treatment do not occur. Further, the occurence of unpredictable side effects is greatly minimized.

The invention provides synthetic peptides that interact with the receptor site of IL-6 or with IL-6 receptors (α and ß) present at target cells.

The invention further provides synthetic peptides that, when combined, interact with the receptor site of IL-6 as well as with IL-6 receptors (α and ß) present at target cells. A mixture of these peptides is particulary valuable as the pharmacological properties of the

peptides can be adjusted to obtain a maximal desired effect. Moreover, half-life time can be prolonged by inserting unnatural amino-acids into the synthetic peptides. The antagonizing or agonizing activity of the peptides is increased by producing di- or multi-meric peptides directed to one or more receptor sites. Such di- or multimeric peptides can for instance be made by linking the peptides via one or more amino-acids such as lysine (Tam, PNAS 1988, 85: 5409-5413). The distribution of the peptides into target organs can be optimized by adjusting the hydrophilic or lipophylic nature of peptides or by binding of these peptides onto peptides that interact with specific organ markers. Finally, the peptides provided can be bound onto the solid phase of membranes or filters that are connected into an extra-corporal blood circulation circuit of the patient. A more efficient clearance of IL-6 and/or soluble IL-6 receptors can in that way be achieved.

Such synthetic peptides can be derived from (A) IL-6, or derived from (B) the receptor α-chain of IL-6 (IL-6Rα, CD126), or from (C) the receptor ß-chain of IL-6 (IL-6Rß, GP130, CD130) and exhibit antagonistic and agonistic activity against the various components and steps of the IL-6 signaling cascade. The peptides were found by testing sets of overlapping amino acid sequences from the published human IL6 (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987), IL-6Ra (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988)) and IL-6Rß (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990)). These overlapping peptides, each twelve amino acids long, were tested in an assay for antagonistic or agonistic IL-6 activity.

The peptides provided by the invention all exhibit antagonistic or agonistic IL-6 activity against the IL-6 signaling cascade as measured in an IL-6 assay. The

peptides of the present invention are too small to generate immune responses. Further, they are too short to contain additional reactive sites, so that the antagonistic and, in addition, the agonistic peptides can advantageously be used to treat patients to counteract and adjust elevated IL-6 levels. The amino acids in all antagonistic or agonistic peptides described below are identified by the one letter code, in which the N-terminal (head) amino acid is listed first (on the left) and the C-terminal (tail) amino acid is listed last (on the right).

A. The antagonistic peptides derived from IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 3 regions that were identified as RYILDGISALRK, STKVLIQFLQKKAKNL, and I-LRSFKEFLQSSLRALRQM.

B. The antagonistic peptides derived from the receptor α-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 3 regions that were identified as QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, and MCVASSVGSKFSKTQTFQGC. The agonistic peptides derived from the receptor α-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following region that was identified as EWGPRSTPSLTTKAVLLVRKFQNSPAED.

C. The antagonistic peptides derived from the receptor ß-chain of IL-6 preferably comprise at least 5 consecutive amino acids selected from the following 4 regions that were identified as PEKPKNLSCIVNEGKKMRCE-WDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, and PVYKVKPNPPHNLSVIN.

Relatively short peptides (as short as a string of 5 amino acids) that are selected from any of the above peptides, or peptides of no more than 30 amino acids long which show antagonistic or agonistic activity as measured in an IL-6 assay and have at least one string of at least 5 amino acids in common with the peptides from groups A,

B or C, are also peptides of the present invention. The peptides according to the invention can vary in length. Also, the peptides comprising a string of at least 5 amino acids which are in common with the peptides from groups A, B, and C can be modified by replacing one or a few amino acids in said string by other amino acids. Such amino acids can be selected from any of the naturally occuring amino acids, but also amino acids that normally do not occur in nature can be used as replacement amino acid. The choice of the replacing amino acid can for example be guided by comparing IL-6 or IL-6 receptor sequences from other species than humans or by selecting amino acids that lead not to extreme functional or conformational changes of the selected peptide, but also other selection methods can be used. More in particular, the present invention relates in a first aspect to a peptide containing at least 5 amino acids and at most 30 amino acids that exhibits antagonistic activity directed against IL-6 and/or against the α-chain of the IL-6 receptor and/or against the ß-chain of the IL-6 receptor.

Also, the present invention relates in another aspect to a peptide containing at least 5 amino acids and at most 30 amino acids that can exhibit antagonistic or agonistic IL-6 activity, depending on the concentration in which it is used. An example of such peptides are peptides selected with as basis with the amino acid sequence EWGPRSTPSLTTKAVLLVRKFQNSPAED as found in the α-chain of the IL-6 receptor. Surprisingly, peptides selected on the basis of the aforementioned sequence expressed antagonistic IL-6 activity at high concentrations whereas at low concentrations a marked agonistic activity was found. Agonistic activity was observed in the in vitro bioassay in a concentration range from 7.5 to 120 μg/ml peptide. At a concentration of ≥120 μg/ml these peptides had an antagonistic effect upon the biological activity of IL-6 in the bioassay. The agonistic peptides can be used in vivo in concentrations

that are relatively equivalent but not necessarily the same as when used in vitro.

Furthermore, the invention provides combinations of peptides, either provided as a simple mixture of several, possibly modified, peptides selected from groups A, B or C, or, provided as, possibly modified, peptides selected from groups A, B, or C that are linked, with direct chemical bonds or using spacer molecules, head to tail, or head to head, or tail to tail, or via side chains of the amino acids present in the selected peptides. Examples of such combinations of peptides are for example using the peptides SLTTKAV and ILRSFKEFLQSS, or WVEAENALGKVTSDH and RYILD, or KAVLLVRK and KAVLLVRK, but many other combinations of two or more peptides can be selected from the peptides listed in groups A, B or C. Such combinations of peptides, be it simple mixtures or bound peptides, can advantageously be used to counteract the events occuring in the IL-6 signaling cascade, such as disrupting the binding of IL-6 to the α-chain by simultanous competing at both the IL-6 and the α-chain binding site, or simultanous competing at the binding sites of the IL-6/α-chain complex and the ß-chain.

The peptides of the invention can suitably be used in a medicinal or pharmaceutical preparation for therapeutic or prophylactic purposes. Further, they can be used in protocols to remove circulating IL-6 from the blood of diseased patients via dialysis methods in which the peptides are bound to a solid phase. Passing blood or blood filtrates along the thus bound peptides will result in clearance of IL-6 that will bind to the peptide at the solid phase. Also the peptides according to the invention may be added to blood or blood filtrates and (ir)-reversibly bind to IL-6 or IL-6 receptor molecules and thus render these inactive before they re-enter the body. Also, the peptides can be used in diagnostic tests, i.e. in direct binding or competition based enzyme-linked immunosorbent assays to measure IL-6 levels.

IL-6 agonistic peptides can completely or partially replace IL-6 that is added to cell-cultures, for example IL-6 is used to grow or culture IL-6 dependant cells, like B-cell hybridomas to which IL-6 as growth factor is often added, bot also cell-cultures in general will benefit from the addition of agonistic IL-6 peptides. The IL-6 agonistic peptides administered to humans or animals can be used to enhance the immune response of an host exposed to a specific immunogenic substance. The IL-6 agonistic peptides can be administered to humans or animals to increase the responsiveness of the immune system of the host. A specific use is in pharmaceutical preparations for topical or intramammary application. When these agonistic peptides are combined with IL-6 antagonists as described, excess of IL-6 can be inhibited without loss of basal IL-6 signal transduction.

Antibodies specifically directed against the peptides, and their corresponding anti-idiotypic antibodies, are part of the invention. Such antibodies can for example be administered to patients treated earlier with the peptides, to counteract the effect of the peptides on the patient. Such antibodies can be used in the above described dialysis protocols and diagnostic tests.

Synthesis of the peptides may be acomplished according to the available methods in the art. The synthesis of the exemplified peptides was done according to Valerio et al. (Int. J. Peptide Res., 42:1-9 (1993) and/or Valerio et al. (Int. J. Peptide Res., 44:148-165 (1994)). Methods for large scale production of syntethic peptides and the purification thereof are well known in the art. The invention is illustrated in the following experimental part.

Experimental part

1. Peptide synthesis.

The peptides of the examples which were intended for identifying active centers in the IL-6 and IL-6 receptor molecules were synthesized using a method according to Valerio et al. (Int. J. Peptide Res., 42:1-9 (1993) and/or Valerio et al. (Int. J. Peptide Res., 44:148-165 (1994)). Multimeric peptides (four branched) were synthetized by the solid-phase method and using of a dispersed system with branching oligolysines as a scaffolding for incorporation of the synthetized antagonistic peptides (Tam, J.P.; Proc. Natl. Acad. Sci. USA, 85:5409-5413 (1988).

2. Proliferation assay to determine antagonistic IL-6 activity.

A set of overlapping peptides, each twelve amino acids long (each consecutive peptide shifts one amino acid, so consecutive peptides have 11 amino acids in common), derived from human IL-6 sequence (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, R., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987), were incubated with cells (B9) at 37°c. After one hour, recombinant human IL-6 (CLB, Amsterdam, The Netherlands) was added at 3 different concentrations (2.5 U/ml, 5 U/ml and 10 U/ml).

A set of overlapping peptides, each twelve amino acids long (each consecutive peptide shifts one amino acid, so consecutive peptides have 11 amino acids in common), derived from human IL6Ra (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988)) or gp130 (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990)), were incubated with 3 different concentrations IL-6 (2.5 U/ml, 5 U/ml, 10 U/ml) diluted in DMEM supplemented with HT for one hour at 37°c. Then the residual IL-6 activity was determined in a biological

assay by measuring the IL-6 dependant proliferative growth of B9 mouse hybridoma cells (Helle, M., Boeije, L., Aarden, L.A.; Eur. J. Immunol. 18:1535-1540 (1988)). Briefly, B9 mouse hybridoma cells were collected during their logarithmic growth phase in IL-6 free media and suspended at a concentration of $1*10^5$ cells/ml in DMEM+HT medium containing 5% FCS. Fifty µl of each IL-6 dilution was combined with each of the synthetized peptides representing IL-6 sequences and incubated for 1 hour at 37°C. This mixture was added in duplicate to 50 µl of the B9 cell suspension in flat-bottommed 96-well tissue culture plates (Greiner) and incubated at 37°c and 5% CO_2 for 72 h. IL-6 activity was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After addition of 25 µl of MTT (5mg/ml dissolved in PBS) to each well and further incubation at 37°c for 4 h, 100 µl of lysis buffer (20% w/v SDS in 50 % dimethyl formamide) was added. Thereafter, incubation was continued over night at 37°c and the next morning absorbance was read at 578 nm.

To determine the agonistic or antagonistic activity to IL-6 of the peptides synthetized from the sequences of the IL-6 receptor a or ß, various concentrations of each of these peptides was combined with 50 µl of the B9 cell suspension ($1*10^5$ cells/ml in DMEM+HT medium containing 5% FCS). This suspension was incubated for 1 hour at 37°C, and combined with each of the dilutions of IL-6 into flat-bottommed 96-well tissue culture plates (Greiner). Plates were incubated at 37°C for 72 h. IL-6 activity was assessed as described above.

Samples without synthetized peptides or with a sham peptide but with IL-6 were used as positive control, whereas samples that contained neither IL-6 nor synthetized peptides were used as negative control.

Inhibition or enhancement of IL-6 activity was determined by calculating the ratio absorbance of test sample and

absorbance positive control both corrected for negative control absorban ce.

3. Toxicity testing of peptides

Three separate test were performed to determine whether the synthetized peptides excert toxic effect in vitro upon erythrocytes (A), or polymorphonuclear cells (B), or hepatocytes (C).

A. Sheep red blood cells (SRBC) were washed five times in PBS. A 1% (v/v) suspension of erythrocytes was prepared in veronal-buffered saline that contained gelatin (GVS: 0.032% gelatin in 3.9 mM barbitone sodium, 1 mM $MgSO_4$, 0.38 mM $CaCl_2$, and 145.6 mM NaCl). Twofold dilutions of the synthetized peptides (50 µl) were made in U-shaped microtiter plates (Greiner Labortechnik) and 50 µl of the SRBC suspension were added to each well. Plates were sealed, mixed and incubated for 2 hours at 37°C. Thereafter, plates were examined for hemolysis. None of the synthetized peptides showed hemolysis.

B. Porcine polymorphonuclear cells (PMN) were isolated from pig blood (Cruijsen, T.L.M., Van Leengoed, L.A.M.G. et al.; Infect. Immun. 60:4867-4871 (1992)). Twofold dilutions of the synthetized peptides (50 µl) were made in flat-bottomed microtiter plates (Greiner Labortechnik) and 50 µl of the PMN suspension ($2*10^6$ cells/ml) were added to each well. Plates were sealed, gently mixed and incubated for 6 hours at 37° and 5% CO_2. Thereafter, plates were examined for cytotoxicity by nigrosine dye exclusion. Non of the synthetized peptides was toxic for PMN.

C. Porcine hepatocytes were isolated from liver of pigs based on Seglens' method (Seglen, P.O.; Methods Cell Biol 13:29-83 (1976)) and adapted according to Monshouwer M., et al. (Toxicol. Applied Pharmacol. in press). Hepatocytes were suspended in Williams' medium E to a concentration of 10^6 cells/ml. From this suspension 1.5 ml was put into each well of 12-well tissue culture plates (Costar) and

incubated for 12 h at 37°C. Adherent hepatocytes were examined for their viability and nonadherent hepatocytes were discarded. Each synthetized peptide was mixed with Williams' medium E (at dilutions of 1:50 and 1:100) and added to wells with adherent hepatocytes. After another 24 h incubation at 37°C viability was assessed by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma). After addition of 1.5 of MTT (1-mg/ml dissolved in Williams' medium E) to each well and further incubation at 37°c for 30 min, 1 ml of lysis buffer (0.8 M HCL in isopropanol) was added. Thereafter, plates were mixed for 10 min and absorbance was read at 560 nm. None of the synthetized peptides proved to affect the viability of the hepatocytes.

4. Effect of IL-6 antagonistic peptides upon IL-6 induced acute phase reaction and downregulation of hepatic biotransformation activities.

Porcine hepatocytes were isolated from liver of pigs based on Seglens' method (Seglen, P.O.; Methods Cell Biol 13:29-83 (1976)) and adapted according to Monshouwer M., et al. (Toxicol. Applied Pharmacol.in press). Hepatocytes were suspended in Williams' medium E to a concentration of 10^6 cells/ml. From this suspension 1 ml was put into each well of 12-well tissue culture plates (Costar) and incubated for 12 h at 37°C. Adherent hepatocytes were examined for their viability and nonadherent hepatocytes were discarded. Each synthetized peptide was mixed (at dilutions of 1:50 and 1:100) with Williams' medium E containing IL-6 (1000 U/ml) and added to wells with adherent hepatocytes. Also a negative (containing no IL-6 and without synthetized peptides in the medium) and positive control (containing 1000 U/ml IL-6 in the medium) were prepared and tested. After an incubation period of 24 hours, the medium was removed and for each well CYP450 dependent enzyme activity of intact monolayers of hepatocytes was determined.

CYP450 enzym assay. CYP450 dependent enzym activity, using testosterone (250μM) as substrate, was determined as previously described by Van 't Klooster et al.(Bioch. Pharmacol.46;1781-1790 (1993)). Briefly, testosteron was mixed with Williams' medium E without fetal calf serum and added to the wells with hepatocytes. After 30 min incubation at 37°C and 5%CO_2, hydroxylated testosteron metabolites in the medium were quantified by HPLC.

HPLC analysis. Aliquots of 1 ml of medium was mixed with 100 μl of a solution of 11ß-testosteron (12,5 μg/ml) in methanol as internal standard and extracted with 5 ml dichlormethane. The organic phase was transferred to clean tubes and evaporated to dryness at roomtemperature under a stream of nitrogen. The residues were dissolved in 130 μl 50% methanol and 20 μl of these solutions were injected for HPLC analysis. The stationary phase consisted of a C18 glasscolumn (20 cm, 3μm particle size, Chrompack, Middelburg, the Netherlands). The mobile phase consisted of buffer A (12% methanol, 75% milli Q water) and buffer B (64% methanol, 6% acetonitril, 30% milli Q water). With these buffers an elution gradient was generated; 10-58% B from 0-45 minutes; 58-59% from 45-50 minutes; 59-10% from 50-53 minutes, with a flow rate of 0,8 ml/min. Metabolites were detected spectrofotometrically at 254 nm. Inhibition of IL-6 dependant downregulation of cytochrome P450 was determined by comparing the relative concentration of hydroxylated testosteron metabolites in medium from adherent hepatocytes incubated with synthetized peptides and IL-6, and the relative concentration of hydroxylated testosteron metabolites in medium from positive and negative control hepatocyte monolayers.

5. Results

Peptides derived from hIL-6, hgp130 (the ß-chain of the IL-6 receptor) and hIL6Ra (the α-chain of the IL-6 receptor) were analysed for antagonistic IL-6 activity.

For hIL-6 peptides, 3 regions were identified which inhibited IL-6 activity in an IL-6 assay (fig. 2). Peptide 31, 119-123 and 167-174 represent the identified regions (RYILDGISALRK, resp. STKVLIQFLQKKAKNL, resp. ILRSFKEFLQSSLRALRQM).

For hIL6Ra, also 3 regions were identified which inhibited IL-6 activity in an IL-6 asay (fig. 3). Peptide 6-9, 24-33 and 80-89 represent the identified regions (QLSCFRKSPLSNVVC, resp. PRSTPSLTTKAVLLVRKFQNS, resp. MCVASSVGSKFSKTQTFQGC).

For hgp130 peptides, 4 regions were identified which inhibited IL-6 activity in an IL-6 assay (fig. 4). Peptide 2-15, 33-46, 73-76 and 92-97 represent the identified regions (PEKPKNLSCIVNEGKKMRCEWDGGR, resp. NFTLKSE-WATHKFADCKAKRDTPTS, resp. WVEAENALGKVTSDH, resp. PVYKVKPNPPHNLSVIN).

The identified peptides with anti-IL-6 activity were not lytic for erythrocytes and not toxic for polymorpho-nuclear cells and not toxic for primary hepatocyte culture cells.

Peptides derived from hIL6Ra (the α-chain of the IL-6 receptor) were analysed for agonistic IL-6 activity and 1 region was identified which stimulated proliferation of B9 cells without IL-6 added to the medium and enhanced IL-6 activity in the B9 bio-assay (fig. 5). Peptide 21-37 represent the region EWGPRSTPSLTTKAVLLVRKFQNSPAED of the IL-6Ra sequence

Agonistic activity was observed in a concentration range from 7.5 to 120 μg/ml peptide. These peptides induced prolife rative growth of the IL-6 dependant cell line B9, and when combined with IL-6 enhanced proliferation of the B9 cell line was examined, and thus the biological activity of IL-6 was enhanced. At a concentration of ≥120 μg/ml these agonistic peptides had an antagonistic effect upon the biological activity of IL-6.

The identified peptides with agonistic IL-6 activity were not lytic for erythrocytes and not toxic for polymorphonuclear cells and not toxic for primary hepatocyte culture cells.

Synthetized peptides from the regions PVYKVKPNPP-HNLSVIN, WVEAENALGKVTSDH, and MCVASSVGSKFSKTQTFQGC inhibit IL-6 regulated downregulation of cytochrome P-450 of hepatocytes.

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CLAIMS

1 A peptide of 5-30 amino acids which peptide exhibits antagonistic activity directed against IL-6.

2 A peptide of 5-30 amino acids which peptide exhibits antagonistic activity directed against the α or β-chain of the IL-6 receptor.

3 A peptide of 5-30 amino acids which peptide exhibits antagonistic or agonistic IL-6 activity.

4 A peptide according to claim 1, 2 or 3 of 5-20 amino acids.

5 A peptide according to claim 4 of 5-12 amino acids.

6 A peptide according to claim 1, 2, 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with one of the following amino acid sequences: STKVLIQFLQKKAKNL, ILRSFKEFLQSSLRALRQM QLSCFRKSPLSNVVC, PRSTPSLTTKAVLLVRKFQNS, MCVASSVGSKFSKTQTFQGC, PEKPKNLSCIVNE-GKKMRCEWDGGR, NFTLKSEWATHKFADCKAKRDTPTS, WVEAENALGKVTSDH, or PVYKVKFNPPHNLSVIN.

7 A peptide according to claim 3, 4 or 5 having at least one string of 5 consecutive amino acids long in common with the following amino acid sequence: EWGPRSTPSLTT-KAVLLVRKFQNSPAED

8 A peptide composition, wherein at least two peptides according to any of claims 1-7 are chemically linked directly or via spacer molecules.

9 A peptide composition according to claim 8 wherein at least two peptides are linked with lysine.

10 A peptide composition wherein at least two peptides according to claim 1, 2, 3, 4, or 5 having at least one string of 5 consecutive amino acids long in common with the amino acid sequence RYILDGISALRK are linked with lysine.

11 A peptide composition according to claim 8, 9 or 10 wherein at least four peptides are linked with branching oligolysines.

12 A mixture comprising peptides and/or peptide compositions according to any of claims 1-11.

13 Antibody specifically directed against a peptide or a peptide composition according to any of claims 1-11.

14 Anti-idiotypic antibody raised against an antibody according to claim 13.

15 A pharmaceutical preparation comprising a peptide or a peptide composition or an antibody according to any of the above claims together with at least one suitable excipient for administration.

16 Use of a pharmaceutical preparation according to claim 15 in the treatment or prevention of an IL-6 related disease.

17 Use of a peptide, peptide composition or antibody according to anyone of claims 1-13 to clear extra-corporeal blood or blood products from IL-6 or IL-6 receptor molecules.

18 A diagnostic assay comprising a peptide or a peptide composition or an antibody according to anyone of claims 1-13.

19 Use of a diagnostic assay according to claim 18 to detect or diagnose IL-6 related disease in man or animals.

20 Use of a peptide according to claim 7 to exert agonistic IL-6 activity at concentrations that are relatively equivalent to 7.5 to 120 µg/ml when tested in vitro in a B9 cell bio assay.

21 Use of a peptide according to claim 20 in cell-culture.

22 A pharmaceutical preparation comprising a peptide according to claim 7 together with at least one suitable excipient for administration.

23 Use of a pharmaceutical preparation according to claim 22 for topical or intra-mammary application.

24 Use of a peptide, or peptide composition according to anyone of claims 1-12 for the manufacture of a medicament for topical or intra-mammary application.

Fig. 1. Amino acid sequences and sources of selected peptides.

Peptides were selected from published sequences of IL-6 (A) and IL-6 a- (B) and ß-receptor (C).

A) human IL6 (Hirano, T., Yasukawa, K., Harada, H., et al.; Nature 324, 73-76 (1986); Yasukawa, K., Hirano, T., Watanabe Y., et al.; EMBO J. 6:2939-2945 (1987)
Amino acid sequence:
APPVPPGEDSKDVAAPHRQPLTSSERIDKQIRYILDGISALRKETCNKSNMCESSK-
EALAENNLNLPKMAEKDGCFQSGFNEETCLVKIITGLLEFEVYLEYLQNRFESSEEQ-
ARAVQMSTKVLIQFLQKKAKNLDAITTPDPTTNASLLTKLQAQNQWLQDMTTH-
LILIRSFKEFLQSSLRALRQM

B) the receptor a-chain of IL-6 (IL-6Ra, CD126), (Yamasaki, K., Taga, T., Hirata, Y., et al.; Science 241:825-828 (1988))
Amino acid sequence:
PPEEPQLSCFRKSPLSNVVCEWGPRSTPSLTTKAVLLVRKFQNSPAEDFQEPCQY-
SQESQKFSCQLAVPEGDSSFYIVSMCVASSVGSKFSKTQTFQGCGILQPDPPANITV

C) the receptor ß-chain of IL-6 (IL-6Rß, GP130, CD130) (Hibi, M., Murakami, M., Saito, M.; Cell 63:1149-1157 (1990))
Amino acid sequence:
PPEKPKNLSCIVNEGKKMRCEWDGGRETHLETNFTLKSEWATHKFADCKAKRDT-
PTSCTVDYSTVYFVNIEVWVEAENALGKVTSDHINFDPVYKVKPNPPHNLSVIN

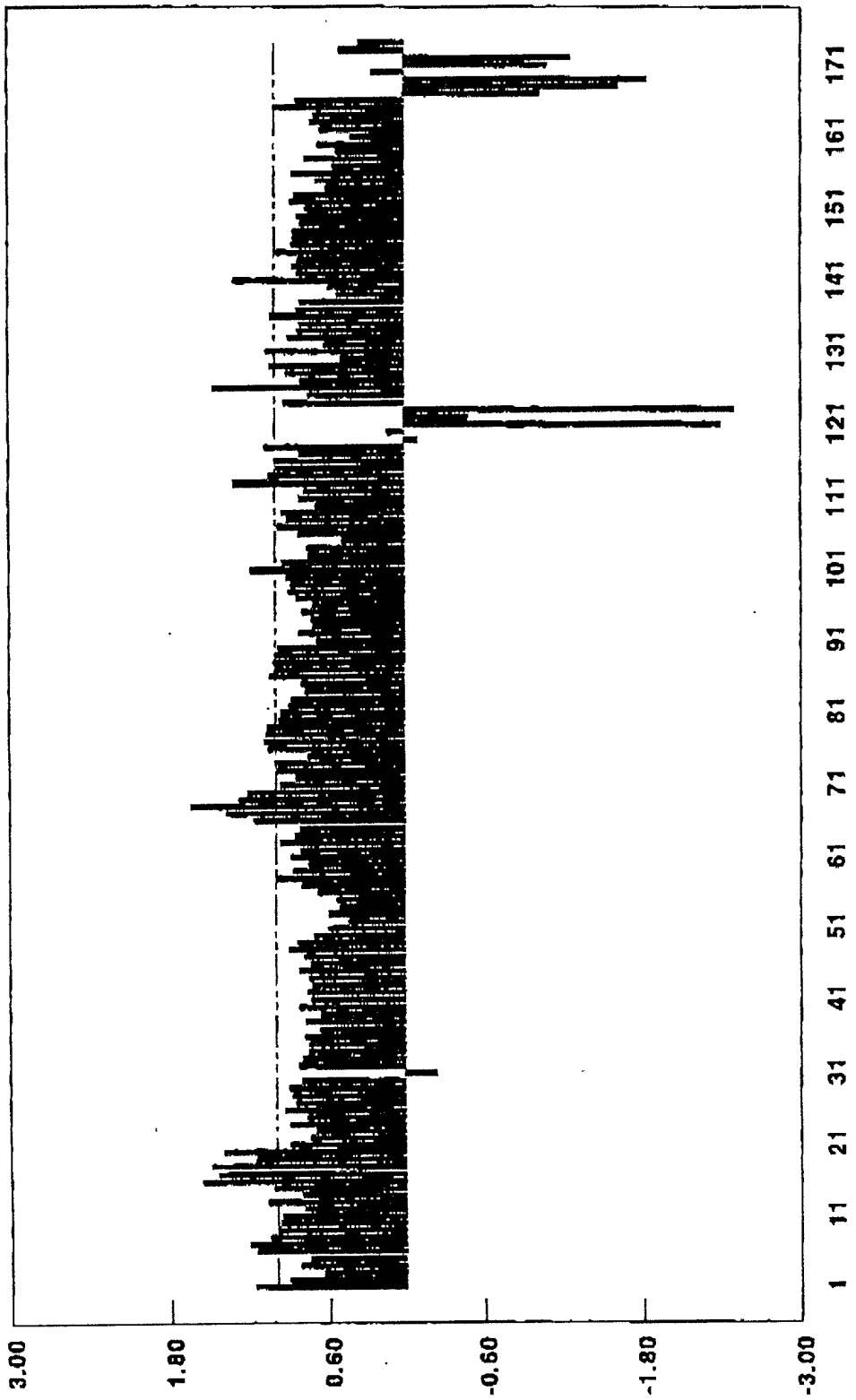

Fig 2: Screening of synthetized peptides, representing IL-6, in the B9 bio-assay

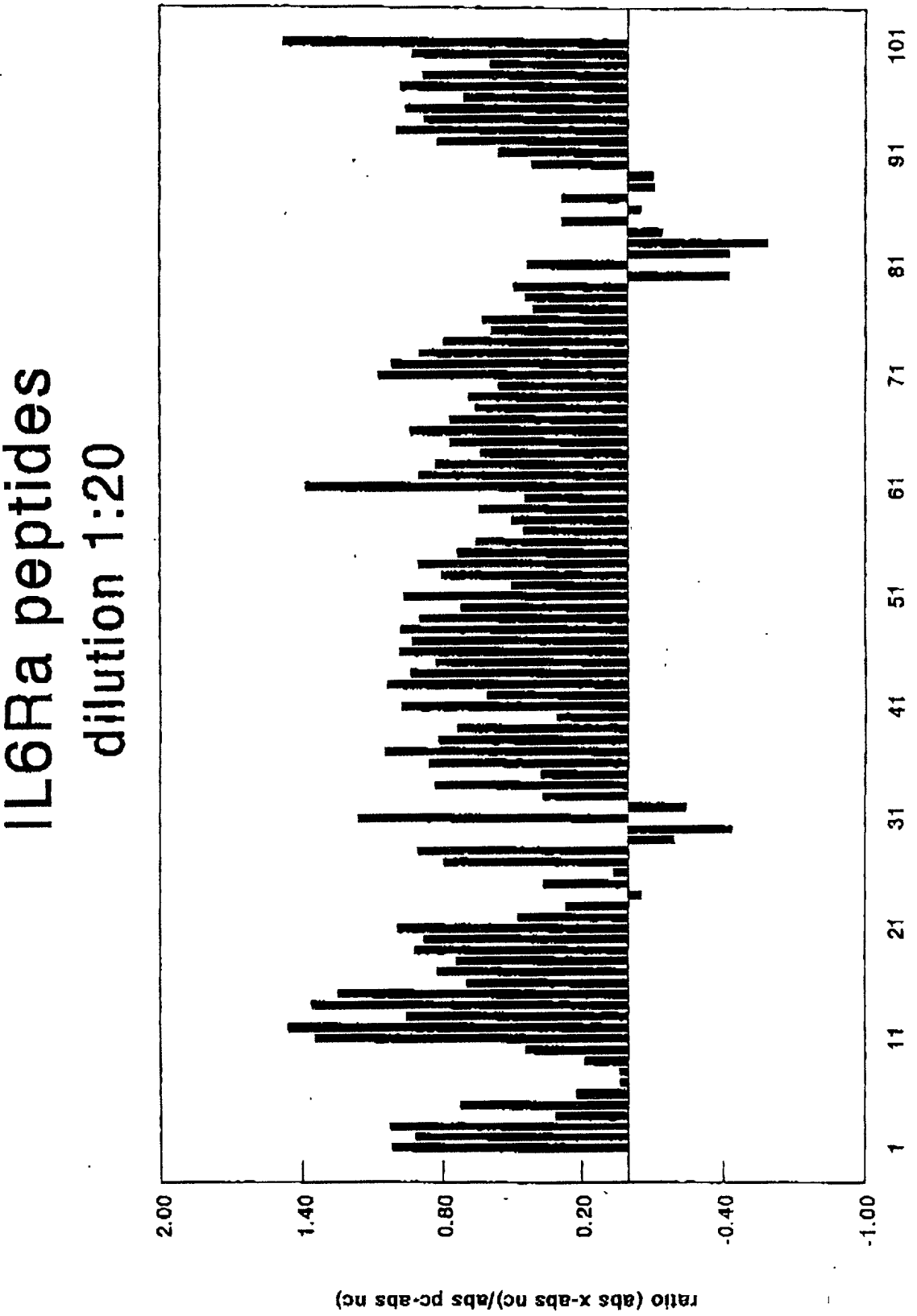

Fig 3: Screening of synthetized peptides, representing IL-6Ra, in the B9 bio-assay

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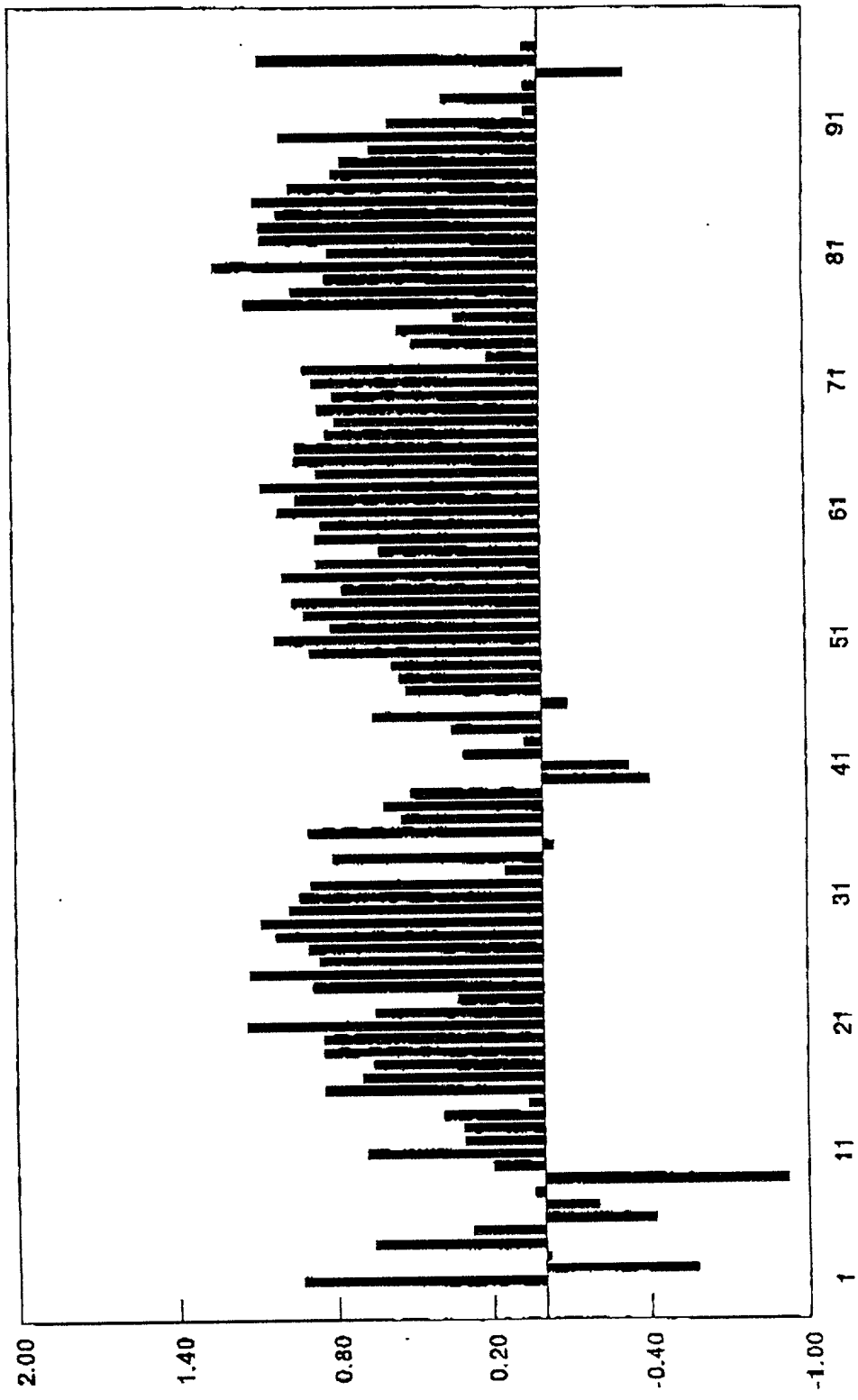

Fig 4: Screening of synthetized peptides, representing gp130, in the B9 bioassay

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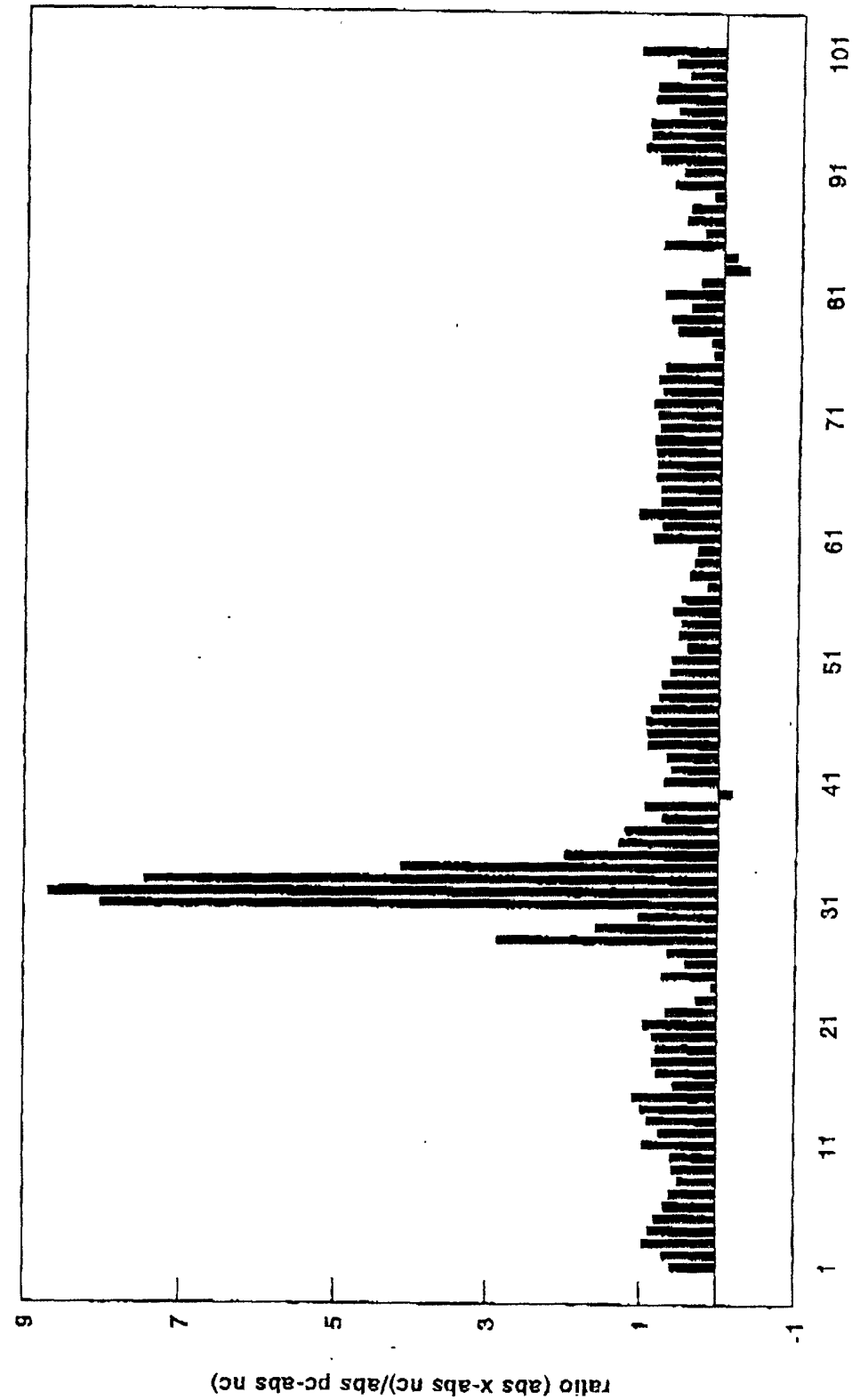

Fig 5: Screening synthetic peptides showing a region with agonistic activity (dilution 1:50)

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DECLARATION AND POWER OF ATTORNEY FOR U.S. PATENT APPLICATIONS

( ) Original ( ) Supplemental ( ) Substitute (X) PCT

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I verily believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Title: IL-6 and IL-6-receptor derived peptides having IL-6 antagonistic or agonistic activity

which is described and claimed in:

( ) the attached specification, or

(X) the specification in the application Serial No. 09/202,104 filed 18 December 1998; and with amendments through _____ (if applicable),

(X) the specification in International Application No. PCT/ NL97/00345, filed 19 June 1997, and as amended on _____ (if applicable).

I hereby state that I have reviewed and understand the content of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge my duty to disclose information of which I am aware which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NO.	DATE OF FILING	PRIORITY CLAIMED
Europe	96201720.8	20 June 1996	(X) YES ( ) NO
			( ) YES ( ) NO
			( ) YES ( ) NO
			( ) YES ( ) NO
			( ) YES ( ) NO
			( ) YES ( ) NO

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

SERIAL NO.	U.S. FILING DATE	STATUS
		( ) Patented ( ) Pending ( ) Abandoned
		( ) Patented ( ) Pending ( ) Abandoned
		( ) Patented ( ) Pending ( ) Abandoned

As a named inventor, I hereby appoint:

as my attorneys to prosecute this application and to transact all business in the United States Patent Office in connection herewith.
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Effective February 27, 1983

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I further declare that all statements made herein of my own knowledge are true, and that all statements on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

st Inventor _____ Date _____

1st Inventor _____ Date January 26th 1999

2nd Inventor _____ Date February 1st 1999

3rd Inventor _____ Date February 5th 1999

5th Inventor _____ Date _____

6th Inventor _____ Date _____